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# United States Patent [19]

Gloger et al.

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[54] **LIPOPROTEIN ASSOCIATED  
 PHOSPHOLIPASE A2 INHIBITORS  
 THEREOF AND USE OF THE SAME IN  
 DIAGNOSIS AND THERAPY**

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[51] Int. Cl.<sup>6</sup> ..... **C12Q 1/68**; C12P 21/06;  
 C12N 15/00; C12N 5/06

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 435/320.1; 435/325; 536/23.1

[58] Field of Search ..... 424/94.1; 435/69.1,  
 435/198, 320.1, 325, 6; 514/44; 536/23.1,  
 24.31, 24.5

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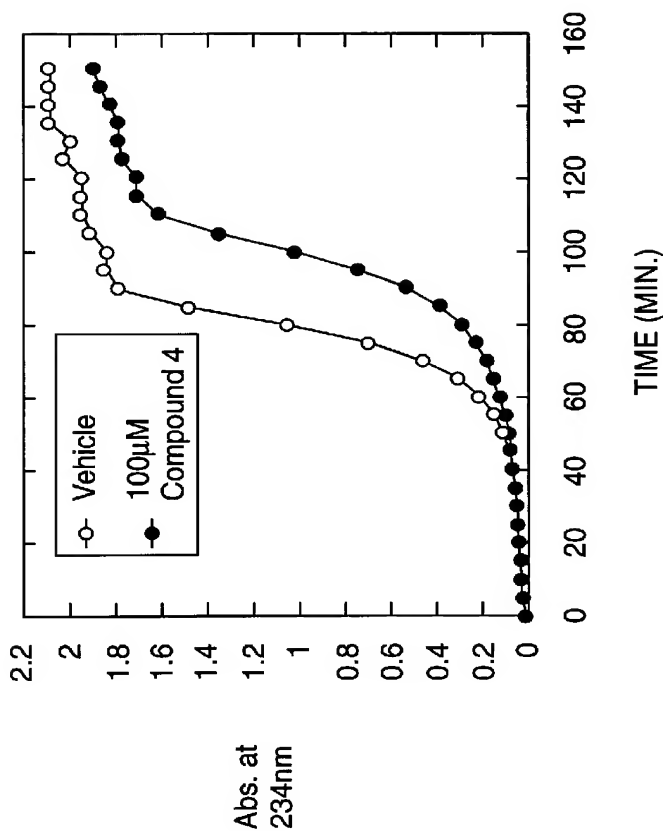
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## [57] ABSTRACT

The enzyme Lp-PLA<sub>2</sub> in purified form, an isolated nucleic acid molecule encoding Lp-PLA<sub>2</sub>, the use of an inhibitor of the enzyme Lp-PLA<sub>2</sub> in therapy and a method of screening compounds to identify those compounds which inhibit the enzyme.

**12 Claims, 2 Drawing Sheets**

FIG. 1



Compound 4 inhibits copper (5 $\mu$ M) - stimulated LDL (150 $\mu$ g/ml) oxidation.

## FIG. 2



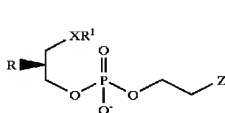
**LIPOPROTEIN ASSOCIATED  
PHOSPHOLIPASE A<sub>2</sub> INHIBITORS  
THEREOF AND USE OF THE SAME IN  
DIAGNOSIS AND THERAPY**

This is a continuation of application Ser. No. 08/387,858, filed Feb. 24, 1995, which is a 371 of PCT/GB94/01374, filed Jun. 24, 1994.

The present invention relates to the use of inhibitors of an enzyme in the therapy, in particular in the treatment of atherosclerosis. The present invention also relates to the isolation and purification of the enzyme, to isolated nucleic acids encoding the enzyme, to recombinant host cells transformed with DNA encoding the enzyme, to the use of the enzyme in diagnosing a patient's susceptibility to atherosclerosis, and to the use of the enzyme in identifying compounds which are potentially useful for the treatment of atherosclerosis.

Lipoprotein Associated Phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>), also previously known in the art as Platelet Activating Factor Acetyl Hydrolase (PAF acetyl hydrolase). During the conversion of LDL to its oxidised form, Lp-PLA<sub>2</sub> is responsible for hydrolysing the sn-2 ester of oxidatively modified phosphatidylcholine to give lyso-phosphatidylcholine and an oxidatively modified fatty acid. Both of these products of Lp-PLA<sub>2</sub> action are potent chemoattractants for circulating monocytes. As such, this enzyme is thought to be responsible for the accumulation of cells loaded with cholesterol ester in the arteries, causing the characteristic 'fatty streak' associated with the early stages of atherosclerosis. Inhibition of the Lp-PLA<sub>2</sub> enzyme would therefore be expected to stop the build up of this fatty streak (by inhibition of the formation of lysophosphatidylcholine), and so be useful in the treatment of atherosclerosis. In addition, it is proposed that Lp-PLA<sub>2</sub> plays a direct role in LDL oxidation. This is due to the poly unsaturated fatty acid-derived lipid peroxide products of Lp-PLA<sub>2</sub> action contributing to and enhancing the overall oxidative process. In keeping with this idea, Lp-PLA<sub>2</sub> inhibitors inhibit LDL oxidation. Lp-PLA<sub>2</sub> inhibitors may therefore have a general application in any disorder that involves lipid peroxidation in conjunction with the enzyme activity, for example in addition to conditions such as atherosclerosis and diabetes other conditions such as rheumatoid arthritis, stroke, myocardial infarction, reperfusion injury and acute and chronic inflammation.

The present invention therefore provides in a first aspect an inhibitor of the enzyme lipoprotein associated Lp-PLA<sub>2</sub> for use in therapy, in particular in the treatment of atherosclerosis. Suitable compounds able to inhibit the Lp-PLA<sub>2</sub> enzyme are known in the art and include for example, the following compounds of structure (I):



in which

R is C<sub>1-6</sub>alkylCONR<sup>2</sup>;

R<sup>2</sup> is hydrogen or C<sub>1-6</sub>alkyl;

X is oxygen, sulphur or —O(CO)—;

R<sup>1</sup> is C<sub>8-20</sub>alkyl;

Z is N(R<sup>3</sup>)<sub>2</sub>, <sup>+</sup>N(R<sup>3</sup>)<sub>3</sub>, SR<sup>3</sup>, <sup>+</sup>S(R<sup>3</sup>)<sub>2</sub>, in which each group R<sup>3</sup> is the same or different and is C<sub>1-6</sub> alkyl, OR<sup>2</sup>, C<sub>1-6</sub>alkanoyl, imidazolyl or N-methylimidazolyl

Suitably R<sup>2</sup> is hydrogen or C<sub>1-6</sub> alkyl; preferably R<sup>2</sup> is hydrogen.

Suitably X is oxygen, sulphur or —O(CO)—; preferably X is oxygen

Suitably R<sup>1</sup> is C<sub>8-20</sub>alkyl; preferably R<sup>1</sup> is C<sub>16-18</sub> alkyl

Suitably Z is N(R<sup>3</sup>)<sub>2</sub>, <sup>+</sup>N(R<sup>3</sup>)<sub>3</sub>, SR<sup>3</sup>, <sup>+</sup>S(R<sup>3</sup>)<sub>2</sub>, in which each group R<sup>3</sup> is the same or different and is C<sub>1-6</sub> alkyl, OR<sup>2</sup>, C<sub>1-6</sub>alkanoyl, imidazolyl or N-methylimidazolyl; preferably Z is SR<sup>3</sup> in which R<sup>3</sup> is methyl or OR<sup>2</sup> in which R<sup>2</sup> is hydrogen

The compounds of structure (I) can be prepared by processes known to those skilled in the art, for example as described in J Chem Soc Chem Comm., 1993, 70-72; J Org Chem, 1983, 48, 1197 and Chem Phys Lipids, 1984, 35, 29-37 or procedures analogous thereto.

When used in therapy, the compounds of structure (I) are formulated in accordance with standard pharmaceutical practice.

The compounds of structure (I) and their pharmaceutically acceptable salts which are active when given orally can be formulated as liquids, for example syrups, suspensions or emulsions, tablets, capsules and, lozenge.

A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in a suitable liquid carrier(s) for example, ethanol, glycerine, non-aqueous solvent, for example polyethylene glycol, oils, or water with a suspending agent, preservative, flavouring or colouring agent.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulation. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and cellulose.

A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule.

Typical parenteral compositions consist of a solution or suspension of the compound or pharmaceutically acceptable salt in a sterile aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil. Alternatively, the solution can be lyophilised and then reconstituted with a suitable solvent just prior to administration.

A typical suppository formulation comprises a compound of formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent such as polymeric glycol, gelatins or cocoa butter or other low melting vegetable or synthetic waxes or fats.

Preferably the composition is in unit dose form such as a tablet or capsule.

Each dosage unit for oral administration contains preferably from 1 to 250 mg (and for parenteral administration contains preferably from 0.1 to 25 mg) of a compound of the formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

The daily dosage regimen for an adult patient may be, for example, an oral dose of between 1 mg and 500 mg, preferably between 1 mg and 250 mg, or an intravenous, subcutaneous, or intramuscular dose of between 0.1 mg and 100 mg, preferably between 0.1 mg and 25 mg, of the compound of the formula (I) or a pharmaceutically acceptable

able salt thereof calculated as the free base, the compound being administered 1 to 4 times per day. Suitably the compounds will be administered for a period of continuous therapy.

The enzyme, lipoprotein associated Lp-PLA<sub>2</sub> has not hitherto been available in isolated purified form. The present invention therefore provides in a further aspect, the enzyme lipoprotein associated Lp-PLA<sub>2</sub> in purified form. By purified form is meant at least 80%, more preferably 90%, still more preferably 95% and most preferably 99% pure with respect to other protein contaminants.

The enzyme Lp-PLA<sub>2</sub> may be characterised by one or more partial peptide sequences selected from SEQ ID NOs:1, 2, 3, 4, 10 and 11 or by the partial peptide sequence comprising residues 271 to 441 or consisting of residues 1 to 441 of SEQ ID NO:9. The enzyme Lp-PLA<sub>2</sub> may further or alternatively characterized by its molecular weight found to be 45 kDa, at least 45 kDa, 45-47 kDa, 46-47 kDa or 45-50 kDa.

The invention also provides fragments of the enzyme having Lp-PLA<sub>2</sub> activity.

The enzyme can be isolated and purified using the methods hereafter described. Once isolated, the protein sequence of the enzyme can be obtained using standard techniques. In identifying said sequence, a number of protein fragments have been identified, each of which comprises part of the whole sequence of the enzyme. These sequences are themselves novel and form a further aspect of the invention.

This invention also provides isolated nucleic acid molecules encoding the enzyme, including mRNAs, DNAs, cDNAs as well as antisense analogs thereof and biologically active and diagnostically or therapeutically useful fragments thereof.

In particular, the invention provides an isolated nucleic acid molecule consisting of bases 1 to 1361 or 38 to 1361 or comprising the sequence corresponding to bases 848 to 1361 of SEQ ID NO:9.

This invention also provides recombinant vectors, such as cloning and expression plasmids useful as reagents in the recombinant production of the enzyme, as well as recombinant prokaryotic and/or eukaryotic host cells comprising the novel nucleic acid sequence.

This invention also provides nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to the novel nucleic acid sequences.

This invention also provides an antisense oligonucleotide having a sequence capable of binding with mRNAs encoding the enzyme so as to prevent the translation of said mRNA.

This invention also provides transgenic non-human animals comprising a nucleic acid molecule encoding the enzyme. Also provided are methods for use of said transgenic animals as models for mutation and SAR (structure/activity relationship) evaluation as well as in drug screens.

This invention further provides a method of screening compounds to identify those compounds which inhibit the enzyme comprising contacting isolated enzyme with a test compound and measuring the rate of turnover of an enzyme substrate as compared with the rate of turnover in the absence of test compound.

"Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide. "Synthetic" polypeptides are those prepared by chemical synthesis.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "double-stranded DNA molecule" refers to the polymeric form of deoxyribonucleotides (bases adenine, guanine, thymine, or cytosine) in a double-stranded helix, both relaxed and supercoiled. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the sense strand of DNA.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide when placed under the control of appropriate regulatory sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes.

DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the expression i.e., the transcription and translation) of a coding sequence in a host cell.

A control sequence "directs the expression" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous DNA sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eukaryotic cells, a stably transformed or transfected cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

Two DNA or polypeptide sequences are "substantially homologous" or "substantially the same" when at least about 85% (preferably at least about 90%, and most preferably at least about 95%) of the nucleotides or amino acids match over a defined length of the molecule and includes allelic variations. As used herein, substantially homologous also

refers to sequences showing identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See e.g., "Current Protocols in Mol. Biol." Vol. I & II, Wiley Interscience, Ausubel et al. (ed.) (1992). Protein sequences that are substantially the same can be identified by proteolytic digestion, gel electrophoresis and microsequencing.

The term "functionally equivalent" intends that the amino acid sequence of the subject protein is one that will exhibit enzymatic activity of the same kind as that of Lp-PLA<sub>2</sub>.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature.

This invention provides an isolated nucleic acid molecule encoding the enzyme Lp-PLA<sub>2</sub>. One means for isolating the coding nucleic acid is to probe a human genomic or cDNA library with a natural or artificially designed probe using art recognized procedures (See for example: "Current Protocols in Molecular Biology", Ausubel, F. M., et al. (eds.) Greene Publishing Assoc. and John Wiley Interscience, New York, 1989,1992); for example using the protein fragment information disclosed herein. The enzyme of this invention may be made by recombinant genetic engineering techniques. The isolated nucleic acids particularly the DNAs can be introduced into expression vectors by operatively linking the DNA to the necessary expression control regions (e.g. regulatory regions) required for gene expression. The vectors can be introduced into the appropriate host cells such as prokaryotic (e.g., bacterial), or eukaryotic (e.g. yeast, insect or mammalian) cells by methods well known in the art (Ausubel et al. supra). The coding sequences for the desired proteins having been prepared or isolated, can be cloned into a suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage  $\lambda$  (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and *Bacillus subtilis*), pBD9 (*Bacillus*), pU61 (*Streptomyces*), pUC6 (*Streptomyces*), Ylp5 (*Saccharomyces*), a baculovirus insect cell system, YCp19 (*Saccharomyces*). See, generally, "DNA Cloning": Vols. I & II, Glover et al., ed. IRL Press Oxford (1985) (1987) and; T. Maniatis et al. ("Molecular Cloning" Cold Spring Harbor Laboratory (1982).

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. The protein sequences of the present invention can be expressed using, for example, the *E. coli* lac promoter or the protein A gene (*spa*) promoter and signal sequence. Leader sequences can be removed by the bacterial host in post-translational processing. See, e.g., U.S. Pat. Nos. 4,431,739; 4,425,437; 4,338,397.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the protein sequences relative to the growth of

the host cell regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the coding sequences may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site. Modification of the coding sequences may also be performed to alter codon usage to suit the chosen host cell, for enhanced expression.

In some cases, it may be desirable to add sequences which cause the secretion of the polypeptide from the host organism, with subsequent cleavage of the secretory signal. It may also be desirable to produce mutants or analogs of the enzyme of interest. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are well known to those skilled in the art. See, e.g., T. Maniatis et al., supra; *DNA Cloning*, Vols. I and II, supra; *Nucleic Acid Hybridization*, supra.

A number of prokaryotic expression vectors are known in the art. See, e.g., U.S. Pat. Nos. 4,578,355; 4,440,859; 4,436,815; 4,431,740; 4,431,739; 4,428,941; 4,425,437; 4,418,149; 4,411,994; 4,366,246; 4,342,832; see also U.K. Patent Applications GB 2,121,054; GB 2,008,123; GB 2,007,675; and European Patent Application 103,395. Yeast expression vectors are also known in the art. See, e.g., U.S. Pat. Nos. 4,446,235; 4,443,539; 4,430,428; see also European Patent Applications 103,409; 100,561; 96,491. pSV2neo (as described in *J. Mol. Appl. Genet.* 1:327-341) which uses the SV40 late promoter to drive expression in mammalian cells or pCDNA1neo, a vector derived from pCDNA1 (*Mol. Cell Biol.* 7:4125-29) which uses the CMV promoter to drive expression. Both these latter two vectors can be employed for transient or stable (using G418 resistance) expression in mammalian cells. Insect cell expression systems, e.g., *Drosophila*, are also useful, see for example, PCT applications U.S. Ser. No. 89/05155 and U.S. Ser. No. 91/06838 as well as EP application 88/304093.3.

Depending on the expression system and host selected, the enzyme of the present invention may be produced by growing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. The protein is then isolated from the host cells and purified. If the expression system secretes the protein into growth media, the protein can be purified directly from the media. If the protein is not secreted, it is

isolated from cell lysates or recovered from the cell membrane fraction. Where the protein is local to the cell surface, whole cells or isolated membranes can be used as an assayable source of the desired gene product. Protein expressed bacterial hosts such as *E. coli* may require isolation from inclusion bodies and refolding. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The identification of this novel target for the treatment of atherosclerosis, also leads to a novel diagnostic method to diagnose a patient's susceptibility to developing atherosclerotic disease.

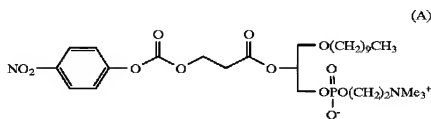
The present invention therefore provides in a still further aspect a diagnostic method comprising isolating a sample of blood from the patient, and assaying said sample for Lp-PLA<sub>2</sub> activity. Patients that are susceptible to atherosclerotic disease are expected to have elevated levels of the Lp-PLA<sub>2</sub> enzyme, and hence the levels of Lp-PLA<sub>2</sub> provides an indication of the patient's susceptibility to atherosclerotic disease. Moreover, Lp-PLA<sub>2</sub> is found located predominantly on dense subfraction(s) of LDL which are known to be very atherogenic. Plasma Lp-PLA<sub>2</sub> levels could therefore provide a ready measure of these very atherogenic small dense LDL particles.

It is expected that the presence of the enzyme in the blood sample of the patient can be assayed by analysis of enzyme activity (i.e. by an assay set up against the purified enzyme as standard); or alternatively by assaying protein content of the sample by using polyclonal or monoclonal antibodies prepared against the purified enzyme. Monoclonal (and polyclonal) antibodies raised against the purified enzyme or fragments thereof are themselves novel and form a further aspect of the invention.

## DATA AND EXAMPLES

### 1. Screen for Lp-PLA<sub>2</sub> Inhibition

Enzyme activity was determined by measuring the rate of turnover of the artificial substrate (A) at 37° C. in 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) buffer containing 150 mM NaCl, pH 7.4.



The dialysed Lp-PLA<sub>2</sub> was submitted to anion exchange chromatography on a mono Q column (Pharmacia) using 50 mM Tris, 10 mM CHAPS, pH=8.0 with a NaCl gradient from 0 to 0.3 M. The Lp-PLA<sub>2</sub> fractions obtained from the mono Q column were applied directly to a Hi Trap Blue cartridge (Pharmacia). The cartridge was washed with 50 mM Tris, 10 mM CHAPS, 0.5 M NaCl, pH=8.0 until the absorbance of the eluate (280 nm) was zero. Lp-PLA<sub>2</sub> was then eluted using 50 mM Tris, 10 mM CHAPS, 1.5 M NaCl, pH=8.0. This gave Lp-PLA<sub>2</sub> which is greater than 95% pure as shown in FIG. 2. This also demonstrates that the native enzyme is extensively glycosylated.

#### 5. Enzyme Sequence

The purity of the final enzyme preparation was verified by five criteria 1) SDS-polyacrylamide gel electrophoresis gave one band for both native and de-glycosylated forms. 2) Reverse phase high pressure liquid chromatography (RP-HPLC) gave a single peak, 3) The intact preparation gave no results by protein sequencing, implying that the protein was N-terminally blocked and free of any contaminants with open N-terminals, 4) By laser desorption mass spectrometry only one broad peak was observed with de-glycosylated protein, and 5) none of the sections of extended peptide data from sequencing gave any database matches indicative of contaminating proteins. Three cleavage strategies were used to obtain internal sequence information; trypsin (after de-glycosylation), cyanogen bromide (methionine cleavage) and BNPS-Skatol (tryptophan cleavage). The resulting peptides were separated by RP-HPLC, collected and sequenced. The accumulated sequence data allowed several extended stretches of primary structure of the Lp-PLA<sub>2</sub> enzyme to be verified. These are shown below as Peptides 1, 2, 3 and 4 (SEQ ID Nos 1 to 4). When searched against the National Centre for Biotechnological information (NCBI) non-redundant peptide sequence databases no high similarity matches were obtained. Estimation of the molecular weight of pure, de-glycosylated protein by laser desorption mass spectrometry gives values in the region of 45–47 kDa (separately 45 kDa and 46–47 kDa), indicating that the sequences constitute approximately 15 to 20% of the protein.

#### 6. Gene Sequence

Three expressed sequence tags (ESTs) from human cDNA libraries have been found to have extensive alignments with the Peptide Sequences 1 to 3. These ESTs are shown below as Nucleotide Sequences 1 to 3 (SEQ ID Nos: 5 to 7) Nucleotide Sequence 1 is a 420 base sequence derived from a human foetal spleen library. Nucleotide Sequence 2 is a 379 base sequence derived from a 12-week human embryo library. Nucleotide Sequence 3 is a 279 base sequence derived from a T-cell lymphoma library. The identities at both the nucleic acid and amino acid level justified an overlapping alignment of the cDNA of all three ESTs, Nucleotide Sequences 3 (bases 1–278), 1 (bases 1–389) (in reverse orientation) and 2 (bases 1–304) with the Peptide Sequences 1, 2 and 3 (partially). Beyond these limits, the poor resolution of the raw sequence data precludes accurate base calling.

There are two remaining unassigned peptide sections from Peptides 3 and 4, both of which are expected to be present in the complete protein, —Q—Y—I—N—P—A—V—, and W—L—M—G—N—I—L—R—L—L—F—G—S—M—T—T—P—A—N—.

#### 7. Isolation of Full-Length Lp-PLA<sub>2</sub> cDNA

The full DNA sequence was determined for the clone (HLTA145) from which the Lymphoma EST (SEQ ID No:7) was derived, giving a total of 572 bases; SEQ ID No:8.

There is one base difference between this sequence and the EST (between bases 1 to 256 of the EST); at position 27 of HLTA145 there is an A compared with a T in the EST. This would cause a coding change; L in HLTA145 compared with F in the EST. Clone HLTA145 was used as a radiolabelled probe to screen the Lymphoma cDNA library in order to isolate the full-length Lp-PLA<sub>2</sub> clone. The library was prepared in the bacteriophage  $\lambda$  vector, Unizap XR (Stratagene).

#### 10 Preparation of the Filters for Screening

The library was plated out at a density of 20,000 plaques per 150 mm petri dish onto *E. coli* XL-1 Blue host cells (ie. 200,000 plaques on 10 dishes). An overnight of XL-1 Blue was prepared in 100 mls LB/0.2% w/v Maltose/10 mM MgSO<sub>4</sub>. The cells were pelleted, resuspended in 50 mls 10 mM MgSO<sub>4</sub> and stored on ice. 180  $\mu$ l of the library bacteriophage stock (23,400 pfu's) were added to 7 mls XL-1 Blue cells, mixed and divided into 10 aliquots of 615  $\mu$ l. The 10 tubes were incubated at 37° C. for 30 minutes. 7 mls of molten ( $\approx$ 45° C.) top agarose (0.7% w/v agarose in LB) were added, mixed well and poured onto 150 mm LB agar plates (1.5% w/v agar in LB). The plates were inverted and incubated at 37° C. for approximately 7.5 hours. The plates were held at 4° C. until needed.

The plaques were transferred to 132 mm Hybond-N nylon filters (Amersham International) by laying the filters on the plates for 2 minutes (4 minutes for the duplicate). The DNA's on the filters were denatured for 2 minutes (0.5 M NaCl, 1.5 M NaOH), neutralised for 4 minutes (1.5 M NaCl, 1.0 M Tris pH7.4) and the filters placed on 2xSSC for 1 minute. The filters were then dried and the DNA cross-linked to the filter using a Stratilinker UV 2400 (Stratagene) at 120,000  $\mu$ Joules/cm<sup>2</sup>.

The filters were pre-hybridised in 1 mM EDTA, 0.5 M NaHPO<sub>4</sub>, 7% SDS (Church, G M. and Gilbert, W. (1984) PNAS USA 81 p1991–1995) in a Techné HB2 hybridisation oven at 55° C. for 3 hours. Each bottle contained 2 filters and 25 mls prehybridization solution.

#### Preparation of the Radiolabelled Probe

The probe cDNA (from HLTA 145) was excised from pBluescript II SK+/- as an approximately 600 bp EcoRI-XhoI fragment and approximately 100 ng of gel purified fragment were labelled using 1.2 MBq <sup>32</sup>P dATP and 1.2 MBq <sup>32</sup>P dCTP by PCR labelling using Taq DNA polymerase (Boehringer Mannheim) and primers designed to prime at the 5' and 3' ends of the EST sequence. The labelling reaction was carried out in a total volume of 200  $\mu$ l and included unlabelled dNTP's at the following concentrations:

dATP 20  $\mu$ M  
dCTP 20  $\mu$ M  
dGTP 200  $\mu$ M  
dTTP 200  $\mu$ M  
The PCR ion was carried out over 35 cycles of:  
94° C. for 30 s  
60° C. for 30 s  
72° C. for 30 s

#### Screening

The radiolabelled probe was denatured at 98° C. for 5 minutes and divided into 10 aliquots of 20  $\mu$ l. One aliquot was added per hybridisation bottle. Hybridization was carried out over 16 hours at 55° C. The filters were washed at 60° C. (2x10 minutes) with 0.1% w/v SDS, 0.1xSSC (50 mls per wash per bottle). The filters were autoradiographed and the films (Fuji Medical X-Ray Film) developed after 5 days exposure.



Duplicate positives were progressed to a secondary screen. The plaques were cored out into 1 ml SM (100 mM NaCl, 10 mM MgSO<sub>4</sub>, 1 M Tris, pH7.4), titrated and plated onto 90 mm petri dishes at between 20 and 200 pfu's per dish. The secondary screen was carried out as described for the primary screen except the filters were washed at 65° C. The autoradiographs were developed after 16 hours exposure.

#### DNA Sequencing

The duplicated positive clones from the secondary screen were excised from the  $\lambda$  Unizap XR bacteriophage vector into the Bluescript phagemid (according to the Stratagene manual) for characterisation. One of the clones, carrying an insert of approximately 1.5 kb, was sequenced on both strands (using the USB Sequenase 2.0 DNA sequencing kit) by primer walking (SEQ ID No:9). The cDNA has an open reading frame with the potential to code for a polypeptide of 441 amino acids.

The 3' region of the full-length cDNA aligns with the HLTA145 sequence with the exception of 3 mismatches (see below). The predicted polypeptide sequence of the lymphoma Lp-PLA<sub>2</sub> is shown as SEQ ID No:9.

Inspection of the full length cDNA (SEQ ID No:9) reveals probable errors in Peptide 3. One of these errors is in the assignment of continuity between V-M which is incompatible with the perfect sequence match with the cDNA after this position. It seems likely that a short peptide, containing the sequence —Q—Y—I—N—P—, co-purified with the longer cyanogen bromide partial cleavage peptide and, by being present in greater quantity, was assigned as the major sequence and contiguous with the subsequent amino acids. The remaining section of Peptide 3 and the whole of Peptide 4 can be identified in the predicted full length enzyme sequence (SEQ ID No:9). It thus seems likely that Peptide 3 is in fact two separate Peptides 5 (SEQ ID No:10) and 6 (SEQ ID No:11). The second probable error has occurred in the transcription from the raw data for Peptide 3 which on checking was consistent with Peptide 5 having the sequence —Q—Y—I—N—P—V—A, rather than Q—Y—I—N—P—A—V—.

The 3 base differences are as follows:

- 1) T at 859 is A in HLTA145; aminoacid change F in full-length, L in HLTA145. (Note that the original EST is identical with the full-length cDNA at position 859).
- 2) C at 1173 is T in HLTA145; aminoacid change A in full-length, V in HLTA145.
- 3) T at 1203 is C in HLTA145; aminoacid change L in full-length, S in HLTA145.

The peptide data and the Foetal Spleen EST sequence (SEQ ID No:5) support the full-length cDNA sequence for differences (2) and (3) although the Human Embryo EST (SEQ ID No:6) is identical to the Lymphoma EST (SEQ ID No:7) at position 1173. The Human Embryo EST (SEQ ID No:6) has a further difference (4) corresponding to position 1245 in the full-length Lymphoma sequence (SEQ ID No:9) (comparison between bases 2 to 304 of the Human Embryo EST and the full-length Lymphoma cDNA).

- 4) A at 1245 is T in the Embryo EST (SEQ ID No:6) (amino acid change D to V in the Embryo EST). Peptide data covering this region supports the Lymphoma DNA sequence (SEQ ID No:9).

The Lp-PLA<sub>2</sub> DNA sequence from 848 to 1361 of SEQ ID No:9 (amino acid residues 271 to 441 of SEQ ID No:9) is the region for which all major data sets agree substantially, i.e. the peptide data, the Foetal spleen, full-length Lymphoma and it includes the known active site and is therefore believed to be a significant characterising region for the Lp-PLA<sub>2</sub> enzyme.

The predicted MW for the full reading frame is 50090. This in excess of that determined for the de-glycosylated, purified protein but post-translational events could account for this discrepancy. The most likely of these are the removal of an N-terminal signal peptide and/or limited proteolytic degradation of the protein C-terminal. The latter could occur in-vivo, during purification, or under the conditions of de-glycosylation.

#### Diagnostic Method

A sample of blood is taken from a patient, the plasma/serum sample prepared and passed through a dextran sulphate column pre-equilibrated with 0.9% (w/v) NaCl solution. Following washes with the same salt solution Lp-PLA<sub>2</sub> is eluted with a 4.1% (w/v) NaCl solution. Heparin agarose columns can also be used with the wash and elution solutions containing 0.7% and 2.9% NaCl, respectively. Enzyme present in the sample is determined by assaying for either

#### (a) Enzyme Activity

The substrate (A) (see structure in 1) is used to assay Lp-PLA<sub>2</sub> activity by monitoring the absorbance change at 400 nm. Purified enzyme is pre-incubated at 37° C. and substrate (50  $\mu$ M) is added after 5 minutes. The absorbance change at 400 nm is monitored for 20 minutes. This substrate has previously been reported as a substrate for classical calcium-dependent PLA<sub>2</sub>s. (Washburn, W. N. and Dennis, E. A., J.Amer Chem.Soc., 1990, 112, 2040–2041); or

#### (b) Protein Content

Total protein content (i.e. enzyme content) can be determined using polyclonal antiserum raised against purified human Lp-PLA<sub>2</sub>. The antiserum recognises both native and glycosylated enzyme as measured by immunoprecipitation of activity and Western Blot analysis.

Polyclonal antiserum was prepared as follows. Immunisation of rabbits involved mixing 0.5 ml of purified human Lp-PLA<sub>2</sub> (=100 $\mu$ g) with an equal volume of Freund's complete adjuvant. The final emulsion was given subcutaneously in 4x0.25 ml injections. Boosting using a Freund's incomplete adjuvant/antigen mixture (4x0.25 ml subcut; dosage=50  $\mu$ g) took place 4 weeks later. Adequate titre was evident at between 6–8 weeks from initial injection.

#### IN THE FIGURES

FIG. 1 is a graph of absorbance at 234 nm against time (min) in a study of inhibition of copper (5  $\mu$ M)—stimulated LDL (150  $\mu$ g/ml) oxidation by compound 4 vs control vehicle.

FIG. 2 is an analysis the purified Lp-PLA<sub>2</sub> material of Example 4 following separation by polyacrylamide gel electrophoresis. Lanes 2, 4 and 6 contain adjacent fractions of purified native Lp-PLA<sub>2</sub>. Lanes 1, 3 and 5 are fractions 2, 4 and 6 respectively after N-deglycosylation.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 11

## (2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 37 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) FRAGMENT TYPE:

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(x) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Leu Lys Leu Lys Gly Asp Ile Asp Ser Asn Ala Ala Ile Asp Leu
1           5           10           15
Ser Asn Lys Ala Ser Leu Ala Phe Leu Gln Lys His Leu Gly Leu His
                20           25           30
Lys Asp Phe Asp Gln
                35
```

## (2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Trp Met Phe Pro Leu Gly Asp Glu Val Tyr Ser Arg Ile Pro Gln Pro
1           5           10           15
Leu Phe Phe Ile Asn Ser Glu Tyr Phe Gln Tyr Pro Ala Asn
                20           25           30
```

## (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Gln Tyr Ile Asn Pro Ala Val Met Ile Thr Ile Arg Gly Ser Val His
1           5           10           15
```

-continued

Gln Asn Phe Ala Asp Phe Thr Phe Ala Thr Gly  
20 25

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (iii) HYPOTHETICAL: NO

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Trp Leu Met Gly Asn Ile Leu Arg Leu Phe Gly Ser Met Thr Thr  
1 5 10 15

Pro Ala Asn

## (2) INFORMATION FOR SEQ ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 420 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AAAAAACCTA TTTTAATCCT AATTGTATTT CTCTATTCCT GAAGAGTCT GTAACATGAT	60
GTGPTGATTG GTTGIGTTAA TGTTGGTCCC TGGAAATAAGA TTCTCATCAT CTCTTCAAT	120
CAAGCAGTCC CACTGATCAA AATCTTTATG AAGTCCTAAA TGCTTTTGTA AGAATGCTAA	180
TGAAGCTTTG TGTCTAAGAT CAATAGCTGC ATTTGAATCT ATGTCTCCCT TTAATTTGAG	240
CATGTGTCCA ATTTATTTGC CAGTNGCAA AGTGAAGTCA GCATAATTCT GGTGGACTGA	300
ACCCCTGATT GTAATCATCT TCTTTCTTT ATCAGGTGAG TAGCATTITT TCATTTTAT	360
GATATTAGCA GGATATTGGA AATATTACAG GTTGNTAAAA AGNGGNGGCT GAGGGATTCT	420

## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 379 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TGCTAATATC ATAAAAATGA AAAAATGCTA CTCACCTGAT AAAGAAAGAA AGATGATTAC	60
AATCAGGGGT TCAGTCCACC AGANTTTTGC TGACTTCACT TTTGCAACTG GCAAAATAAT	120
TGGACACATG CTCAAATTTAA AGGGAGACAT AGATTCAAAT GTAGCTATTG ATCTTAGCAA	180
CAAGCTTCA TTACGATTCT TACAAAAGCA TTAGGACTT CATAAAGATT TTGTTCACTG	240

-continued

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GGACTGCTTG ATTGAAGCAG ATGATGAGAA TCTTATTC	300
CAATTCACCA CATCATGTTT ACAGAACTTC TTCCAGGGAA TAGGAGGAAA TACAATTGGG	360
GTTTAAAAATA GGTTTTTTTT	379

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 279 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GAAGAATGCA TTAGATTAA AGTTTGATAT GGAACAAC	60
AAAAATAGCA CTAATTGCAC ATTCTTTTGG TGGACCAACG GTTATTCAGA CTCTTAGTCA	120
AGATCAGAGA TTCAGATGTG GTATTGCCCT GGTGCAATGG ATGTTTCCAC TGGGTGATGA	180
AGTATATTCC AGAATTCCTC AGCCCCCTCT TTTATCAAC TCTGAATATT TCCAATATCC	240
TGCTAATATC ATAAAAATGG AAAAATGCTA CTCACCTGG	279

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 572 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AAATAGCAG TAATTGGACA TTCTTTAGGT GGAGCAACGG TTATTCAGAC TCTTAGTGAA	60
GATCAGAGAT TCAGATGTGG TATTGCCCTG GATGCAATGA TGTTCCACT GGGTGATGAA	120
GTATATTCCA GAATTCCTCA GCCCTCTTIT TTTATCAACT CTGAATATTT CCAATATCCT	180
GCTAATATCA TAAAAATGAA AAAATGCTAC TCACCTGATA AAGAAAGAAA GATGATTACA	240
ATCAGGGGTT CAGTCCACCA GAATTTTGCT GACTTCACCT TTGCAACTGG CAAATAATT	300
GGACACATGC TCAAAATTAA GGGAGACATA GATTCAAATG TAGCTATTGA TCTTAGCAAC	360
AAAGCTTCAT CAGCATCTT ACAAAGCAT TTAGGACTTC ATAAAGATTT TGATCAGTGG	420
GACTGCTTGA TTGAAGCAGA TCATGAGAAT CTTATTCAG GGACCAACAT TAACACAACC	480
AATCAACACA TCATGTTACA GAACTCTTCA GGAATAGAGA AATACAATTA GGATTAAAT	540
AGGTTTTTTA AAAAAAAAAA AAAAAAACT CG	572

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1361 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA



-continued

Asp Arg Glu Lys Ile Ala Val Ile Gly His Ser Phe Gly Gly Ala Thr	
265 270 275	
GTT AIT CAG ACT CTT ACT GAA GAT CAG AGA TTC AGA TGT GGT AIT GCC	919
Val Ile Gln Thr Leu Ser Glu Asp Gln Arg Phe Arg Cys Gly Ile Ala	
280 285 290	
CTG GAT GCA TGG ATG TTT CCA CTG GGT GAT GAA GTA TAT TCC AGA ATT	967
Leu Asp Ala Trp Met Phe Pro Leu Gly Asp Glu Val Tyr Ser Arg Ile	
295 300 305 310	
CCT CAG CCC CTC TTT TTT ATC AAC TCT GAA TAT TTC CAA TAT CCT GCT	1015
Pro Gln Pro Leu Phe Phe Ile Asn Ser Glu Tyr Phe Gln Tyr Pro Ala	
315 320 325	
AAT AIC ATA AAA ATG AAA AAA TGC TAC TCA CCT GAT AAA GAA AGA AAG	1063
Asn Ile Ile Lys Met Lys Lys Cys Tyr Ser Pro Asp Lys Glu Arg Lys	
330 335 340	
ATG AIT ACA ATC AGG GGT TCA GTC CAC CAG AAT TTT GCT GAC TTC ACT	1111
Met Ile Thr Ile Arg Gly Ser Val His Gln Asn Phe Ala Asp Phe Thr	
345 350 355	
TTT GCA ACT GGC AAA ATA ATT GGA CAC ATG CTC AAA TTA AAG GGA GAC	1159
Phe Ala Thr Gly Lys Ile Ile Gly His Met Leu Lys Leu Lys Gly Asp	
360 365 370	
ATA GAT TCA AAT GCA GCT ATT GAT CTT AGC AAC AAA GCT TCA TTA GCA	1207
Ile Asp Ser Asn Ala Ala Ile Asp Leu Ser Asn Lys Ala Ser Leu Ala	
375 380 385 390	
TTC TTA CAA AAG CAT TTA GGA CTT CAT AAA GAT TTT GAT CAG TGG GAC	1255
Phe Leu Gln Lys His Leu Gly Leu His Lys Asp Phe Asp Gln Trp Asp	
395 400 405	
TGC TTG ATT GAA GGA GAT GAT GAG AAT CTT ATT CCA GGG ACC AAC ATT	1303
Cys Leu Ile Glu Gly Asp Asp Glu Asn Leu Ile Pro Gly Thr Asn Ile	
410 415 420	
AAC ACA ACC AAT CAA CAC ATC ATG TTA CAG AAC TCT TCA GGA ATA GAG	1351
Asn Thr Thr Asn Gln His Ile Met Leu Gln Asn Ser Ser Gly Ile Glu	
425 430 435	
AAA TAC AAT T	1361
Lys Tyr Asn	
440	

## (2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 7 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Gln Tyr Ile Asn Pro Val Ala  
 1 5

## (2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met	Ile	Thr	Ile	Arg	Gly	Ser	Val	His	Gln	Asn	Phe	Ala	Asp	Phe	Thr
1				5					10					15	
Phe	Ala	Thr	Gly												
			20												

We claim:

1. An isolated nucleic acid molecule encoding an enzyme fragment having the sequence corresponding to: SEQ. ID Nos: 1; 2; 3; 4; 10; or 11.

2. An isolated nucleic acid molecule encoding Lp-PLA<sub>2</sub> and having the sequence corresponding to:

bases 1-389 of SEQ. ID NO:5;

bases 1-304 of SEQ. ID NO:6;

bases 1-278 of SEQ. ID NO:7; or

SEQ. ID NO:8.

3. An isolated nucleic acid molecule encoding Lp-PLA<sub>2</sub> and having the sequence corresponding to bases 848 to 1361 of SEQ. ID NO:9.

4. An isolated nucleic acid molecule consisting of bases 1 to 1361 or 38 to 1361 of SEQ. ID NO:9.

5. A recombinant vector comprising the nucleic acid molecule of claim 1.

6. A host cell comprising the molecule of claim 1.

7. A recombinant vector comprising the nucleic acid molecule of claim 2.

8. A host cell comprising the molecule of claim 2.

9. A recombinant vector comprising the nucleic acid molecule of claim 3.

10. A host cell comprising the molecule of claim 3.

11. A recombinant vector comprising the nucleic acid molecule of claim 4.

12. A host cell comprising the molecule of claim 4.

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